THE INFLUENCE OF FUNCTIONALIZED SBA-15 ON THE PRODUCTION OF CITRONELLOL ESTER VIA IMMOBILIZED *CANDIDA RUGOSA* LIPASE

YASMIN BINTI CHE ANI

UNIVERSITI SAINS MALAYSIA

2016

THE INFLUENCE OF FUNCTIONALIZED SBA-15 ON THE PRODUCTION OF CITRONELLOL ESTER VIA IMMOBILIZED *CANDIDA RUGOSA* LIPASE

by

YASMIN BINTI CHE ANI

Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

February 2016

ACKNOWLEDGEMENTS

In the name of Allah, the Most Beneficent and the Most Merciful. Praise be to Allah SWT Lord of the world for allowing me to complete this research work.

I wish to acknowledge my sincere gratitude to my supervisor and also as a Dean School of Chemical Engineering, Professor Dr. Azlina Harun@Kamaruddin and my co-supervisor and also as Deputy Dean (Research) Professor Dr. Ahmad Zuhairi Abdullah who has shown endless support, understanding and kindly helped me to complete my thesis. Indeed, their kindness, understanding, and help for the success of this thesis are greatly indebted. Thanks also to Deputy Dean (Academic) Associate Professor Dr. Mohamad Zailani Abu Bakar, Program Chairman School of Chemical Engineering (Infrastructure Management), Associate Professor Dr. Tan Soon Huat, Director of Research Creativity and Management Office (RCMO), Professor Dr. Lee Keat Teong, Professor Dr. Abdul Rahman bin Mohamed and all the lecturers at School of Chemical Engineering for their guidance and support.

I also would like to express my gratitude for extraordinary effort from all the technicians, especially Mr. Mohd Faiza Ismail, Mrs. Latifah Abd. Latif, Mr. Muhamad Ismail Abu Talib, Mrs. Nur Ain Natasya Shaari, Mr. Shamsul Hidayat Sharan, Mr. Muhd Arif Mat Husin, Mrs. Nor Zalilah Ahmad, Mrs. Noraswani Muhamad, Mrs. Yusnadia Mohd. Yusof, Mr. Mohd Roqib Mohd Rashidi, Mr. Syed Nor Izwan Said Muliyana, Mr. Nurnajib Che Aziz, Mr.Osmarizal Osman, Mr. Mohd Rasydan Omar, Mr. Shaharin Mohamed, Mr. Aziz Ismail, Mr. Said Saidin, Mr. Mohd Yusof Ismail, Mrs. Rosni Mohamad Yusof and Mr. Mohd. Hisyam Adnan.

Not forgetting lab technicians in the main campus, Mr. Masrul Mansur from School of Biological Sciences and Mr. Sivaraj a/l Panirselvam from School of Chemical Sciences.

I also wish to acknowledge the professional work of the management staff at School of Chemical Engineering, especially to Mr. Samsuri Musa, Mr. Mokhtar Alfakari Anurbek, Mrs. Siti Maryam Ibrahim, Mrs. Aniza Abd. Ghani, Mrs. Nor Ain, Mrs. Esah Abdul Manaf, Mrs. Normie Hana A. Rahim, Mr. Noorazmi Md. Tahir, Mr. Ahmad Yazid Yahya, Mrs. Hasmah Muhammad Yadzi and also to ex-staff of School of Chemical Engineering, Ms. Badilah Baharom, Mrs. Azizah Embi, Mrs. Azni Shahida Khalid and Mrs. Sharida Sajili.

I also would like to express my gratitude to all my friends especially Aziah, Tijah, Nora, Jus, Syura, Mizah, Sim, Umi, Zali, Aireene, Jiah, Mariani, Zira, Rahmah, Eka, Pak Joni, Hafiz, Siti Fatimah, Siti Nur, Ila, Syu, Dahlia, Ida, Azam, Fadzil, Fatihah, Nurul, Areen, Sariyah, Zarith, Dayah, Fizah, Azie, Aini, Hana, Chikin, Aiiin, Wani and to all people that contributed their helps directly or indirectly by helping me finishing my thesis, thank you very much.

I am also thankful to the Ministry of Higher Education (MOHE) for providing the Fundamental Research Grant Scheme (FRGS) and National Science Fund Scholarship from Ministry of Science, Technology and Innovation **(**MOSTI**)**. I also would like to thank Universiti Sains Malaysia for providing the PRGS RU GRANT.

This thesis is dedicated to my parents, Che Ani Abdullah and Che Olah Hanapiah and to my lovely brothers Rizal and Jasri. Thank you for your prayers, patience and full support that gave me the courage to go through all the obstacles to complete my thesis.

Lastly, I also would like to dedicate my thesis to my late friend Nor Azini Aziz (Gini), my late aunt Cik Solehah and Professor Mashitah Mat Don. May Allah SWT have mercy on them.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

2D	2-dimensional
APTES	3-aminopropyltriethoxysilane
APTMS	3-aminopropyltrimethoxysilane
BCA	Bicinchoninic Acid
BET	Brunauer, Emmet and Teller
BJH	Barrett-Joyner-Halenda
BSA	Bovine serum albumin
CALB	*Candida antartica* lipase B
CRL	*Candida rugosa* lipase
EA	Elemental Analysis
EDX	Energy Dispersive X-ray
FID	Flame Ionization Detector
FSM	Folded Sheet Material
FT-IR	Fourier Transform Infrared
GC	Gas Chromatography
HCl	Hydrochloric acid
hrs	Hours
i.e.	id est which means that is
K	Kelvin
KBr	Potassium bromide
KH_2PO_4	Potassium dihydrogen phosphate
KSW	Kanemite Sheets performed at Waseda University
LYS	Lysozyme

MCF	Mesostructured cellular foam
mM	mili Molar
MOMD	manganese-oxides-modified diatomite
MPTMS	3-mercaptopropyltrimethoxysilane
MSU	Michigan state University
MW	Molecular weight
MWCNTs	multi-walled carbon nano-tubes
Na_2CO_3	Sodium carbonate
$NaHCO_3$	Sodium bicarbonate
$Na_2HPO_4.2H_2O$	Disodium hydrogen phosphate
NaOH	Sodium hydroxide
nm	nanometer
P123	Pluronic P123
PEO	polyethylene oxide
PGA	penicillin G acylase
PPL	porcine pancreas lipase
PPO	polypropylene oxide
PXRD	Powder X-Ray Diffraction
rpm	rotations per minute
RSMS	Rod shape mesoporous silica
SBA	Santa Barbara Amorphous
SEM	Scanning Electron Microscopy
SiO_2	Silica dioxide
TCP	Tri block Co Polymer
TEM	Transmission Electron Microscopy

TEOS	Tetraethylorthosilicate
TGA	Thermogravimetric Analysis
UV	Ultraviolet
vs	versus
wt	weight
XRD	X-ray Diffraction

LIST OF SYMBOLS

a_L	Langmuir isotherm constant
[A]	Concentration of substrate A
[B]	Concentration of substrate B
B_T	Temkin isotherm constant related to the heat of adsorption
b_T	Temkin isotherm constant
C_o	The highest initial concentration of *Candida rugosa* lipase
C_e	The equilibrium concentration of *Candida rugosa* lipase
C_i	The initial concentration of *Candida rugosa* lipase
C_t	The concentration of *Candida rugosa* lipase at time, *t*
E_a	Activation energy
K_f	Freundlich isotherm constant
K_A	Michaelis-Menten constant for citronellol
K_B	Michaelis-Menten constant for octanoic acid
K_i	Inhibition constant for octanoic acid
K_L	Langmuir isotherm constant
K_M	Michaelis constant
$K_M{}^A$	Michaelis constant for substrate A
$K_M{}^B$	Michaelis constant for substrate B
$K_S{}^A$	Dissociation constant for substrate A
K_T	Equilibrium binding constant corresponding to the maximum binding energy (ΔG_{max})
k_1	Equilibrium rate constant of pseudo-first sorption
k_2	Equilibrium rate constant of pseudo-second sorption
n	Adsorption tendency

q_e	The amount of *Candida rugosa* lipase adsorbed at equilibrium
q_t	The amount of *Candida rugosa* lipase adsorbed at time, t
R	Universal Gas constant
R_L	Dimensionless constant separation factor
R^2	Correlation coefficient
T	Absolute temperature
t	Time
v_o	Initial velocity
V	The volume of the phosphate buffer pH 8.0 solution
V_{max}	Maximum velocity
W	The weight of mesoporous silica support
ΔG	Gibbs free energy
ΔH	Enthalpy
ΔS	Entropy
Δq	Normalized standard deviation
λ	Wavelength

PENGARUH SBA-15 BERFUNGSI DALAM PENGHASILAN SITRONELLOL ESTER MELALUI LIPASE *CANDIDA RUGOSA* TERSEKATGERAK

ABSTRAK

Pengesteran enzim menggunakan lipase terbukti mempunyai potensi dalam penghasilan ester. Ini adalah kerana berkurangnya sumber asli yang digunakan dalam penghasilan ester. Sebagai alternatif, sintetik ester yang mempunyai ciri-ciri yang sama dengan bahan alam semulajadi telah dihasilkan. Dalam kajian ini, sitronellil oktanoat ester dikaji sebagai reaksi model.

Lipase tersekatgerak sangat penting dalam proses pengesteran kerana ia boleh diguna semula, proses operasi yang lebih fleksibel dan pemerolehan semula produk dari lipase yang lebih mudah. Oleh itu, SBA-15 berliang meso tulen telah digunakan sebagai sokongan untuk enzim tersekatgerak kerana saiz liang yang lebih besar dan kestabilan haba yang lebih baik. Manakala kaedah bukan hidroterma yang akan menghasilkan ciri-ciri SBA-15 yang sama dengan kaedah hidroterma telah dihasilkan dalam kajian ini. Keadaan terbaik untuk sintesis SBA-15 adalah pada suhu 40 ^{o}C, 2.5 M HCl dan 1.79:1 nisbah molar TEOS/TCP yang menghasilkan luas permukaan BET tertinggi iaitu masing-masing 644.14 m^2/g, 661.17 m^2/g dan 641.35 m^2/g. SBA-15 tulen juga berfungsi dengan kumpulan fungsi amina secara sintesis berasingan dalam keadaan refluks pada suhu 85 ^{o}C selama 2 jam. Keadaan yang terbaik untuk menyekatgerak SBA-15 tulen dan APTES-SBA-15 dengan *Candida rugosa* lipase (CRL) adalah pada aktiviti enzim 19530 U/mg, pH bufer 8.0 dan pada suhu 35 ^{o}C yang memberikan peratusan jumlah enzim terjerap yang tinggi iaitu kira-kira 95 % .